EFFECTS OF CULTURAL VARIATION ON CELL SIZE IN A MARINE FUNGUS[1]

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Tests for the determination of tolerance ranges of fungi isolated from salt water have indicated that some varieties show, in laboratory culture, a definite reaction to salinity and temperature, and especially to a combination of the two factors. These organisms will grow in media containing salts in about the same concentration as that found in the sea, and at temperatures they might meet in nature. When incubated at sub-optimal temperatures, they grow faster in less concentrated salts, or at supra-optimal temperatures they grow faster in more concentrated salt media.

Interdependence of salinity and temperature has been noted in a number of organisms, with a variety of criteria being used by different investigators. Johnson (1960) used infectivity of a fungus on a barnacle. Todd and Dehnel (1960) used high-temperature resistance in crabs, as McLeese (1956) did in the lobster. In all these studies, a high salinity coupled with high temperature proved favorable. An exception is the work of Broekema (1941), who, using survival time as a criterion, found that shrimp endured a low temperature better if the salinity was high.

Conclusions on the salinity-temperature relations of fungi were based, in our work, on growth rates, with growth measured as increase in colony diameter (Ritchie, 1957). We considered growth in hyphal length to be proportional to total growth of the colony, but a question arises as to whether variations in total growth of these lower forms are reflected in the sizes of individual cells. Consequently, a series of experiments, set up so as to allow for variation of both temperature and salinity, was conducted to give information on how these factors affect the cell size of a sensitive organism.

Materials and Methods

The fungus used in the present experiments was a variety of *Phoma herbarum* West., an imperfect form isolated from yellow pine panels which had been submerged in Limon Bay, Panama. It was cultivated on agar media containing 0.5% glucose, 0.1% Difco yeast extract, and sea water which was either diluted with distilled water or concentrated by evaporation to produce a graded series containing 8, 15, 23, 30, 60, and 90 parts per thousand of salts. Cultures were prepared in triplicate, and the series at all salinities was incubated at the following temperatures: 7° C., 16° C., 25° C., 30° C., and 37° C. Figure 1 shows the results of a preliminary experiment, which indicated that at an incubation temperature of 16° C., the fungus would grow fastest in a salt concentration of about

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20 S ‰. At 25° C., the "optimum" was about 25 S ‰; at 30° C. about 38 S ‰; and at 37° C. about 48 S ‰.

Using these data as a guide, we ran two sets of cultures, one with the temperature varied as before, but with the media made up to the salt concentration which seemed optimal for each temperature, and a second set, again with the same temperatures, but with all the media made to contain 25 parts per thousand of salts. The latter concentration was chosen because, being one which will support some growth at any temperature, it would permit comparison of various temperatures with uniform salinity.

After five days of incubation, samples were cut from the colonies just back of the advancing edge of the mycelium, where the hyphae were no longer growing.

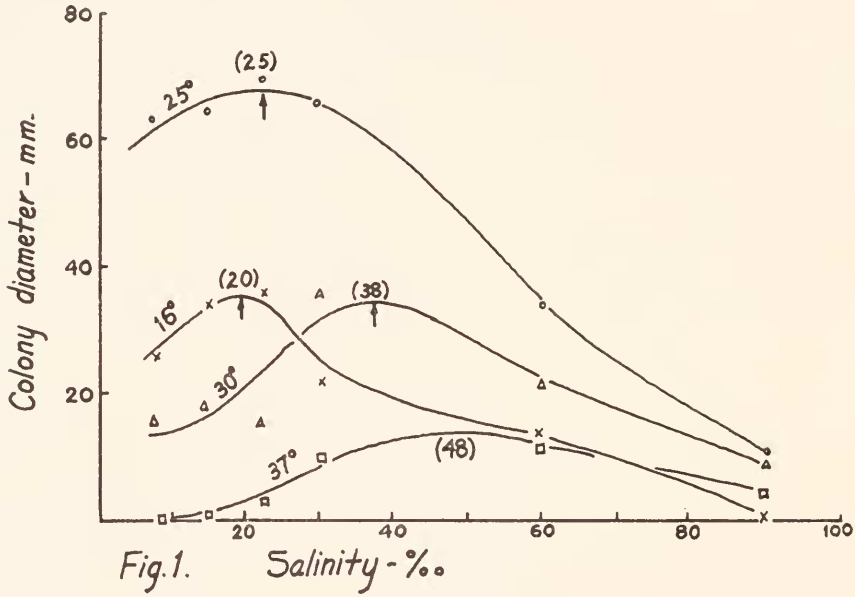

FIGURE 1. Effect of temperature and salinity on total growth of *Phoma herbarum*. The numbers in parentheses at the peak of each curve indicate the approximate salinity optimum for each temperature.

Hyphal diameters were measured with an ocular micrometer in conjunction with an oil immersion objective. Measurements were estimated to the nearest half unit of the micrometer. In any field of view, randomly picked, hyphae were measured as they fell across the micrometer scale, with subjective choice minimized as much as possible. In the experiment with salinity at 25 S ‰, fifty measurements were made on each culture; in the second, when salinity was varied, one hundred measurements were made. In one instance (25° C. and 25 S ‰), three independent, fifty-count measurements were made in order to find whether important sampling errors would show up. Inasmuch as the three runs gave consistently similar data, the number fifty was considered adequate for a sample.

Diameters only were used, and cell lengths were ignored because they are extremely variable and irregular.

The figures from any one run, as for example from 30° C. and 38 S ‰, were arranged in order, and the number of identical hyphae grouped together and counted. The resulting number was expressed as a per cent of the total count for that run, and the frequency was plotted as the ordinate, with hyphal diameters on the abscissa. Curves could thus be made comparing temperatures or salinities separately or simultaneously.

Results and Conclusions

Of the temperatures tried, the best growth of this isolant of *Phoma* occurred at 25° C. Growth at higher temperature (30° C.) or lower (16° C.) was markedly

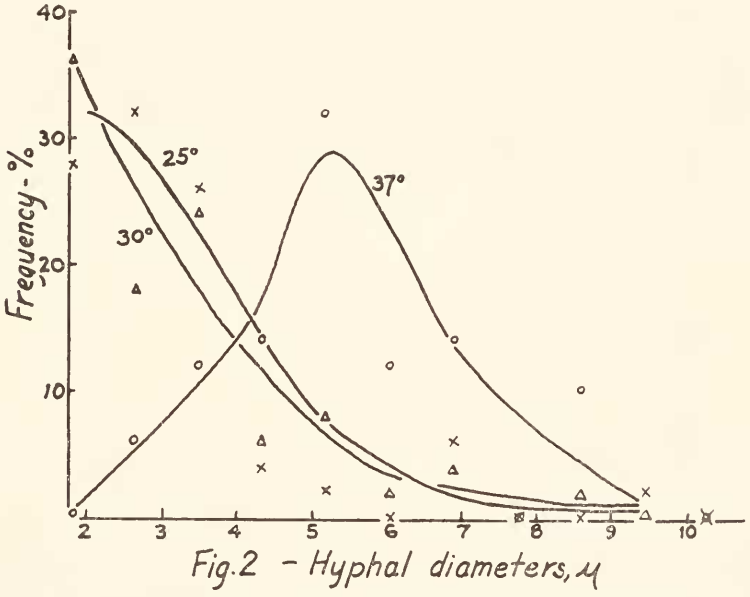

Figure 2. Effect of temperature on hyphal diameter. Percentage distribution of hyphal diameters in microns. Temperatures as indicated; all media containing 25 S ‰.

less than at the optimum, while at 37° C. it was severely retarded (Fig. 1). When cell size is considered, diameters of hyphae were, within limits, little affected by changes in temperature. Measurements from cultures incubated at 25° C. and 30° C. yielded curves that are similar (Fig. 2). Actually, the curve from the 16° C. culture is about the same, but was omitted from the graph in the interest of visual simplicity. But when the temperature was maintained at the rather high level of 37° C. with salinity kept low, the shape of the curve was altered, with a much larger percentage of thicker hyphae and a reduction in the number of slender ones. In fact, only when a definitely supra-optimal temperature was applied did the curve approach what would be expected of a normal size distribution, statistically speaking.

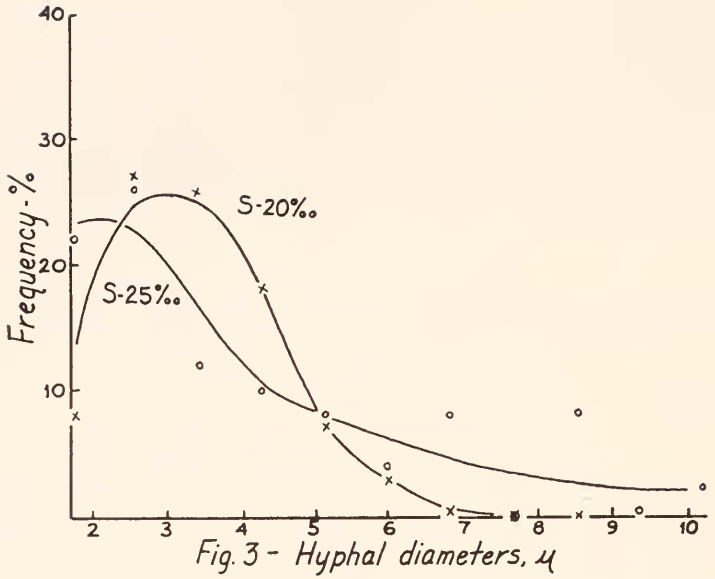

FIGURE 3. Effect of salinity on hyphal diameter. Salinities as indicated; both cultures at 16° C.

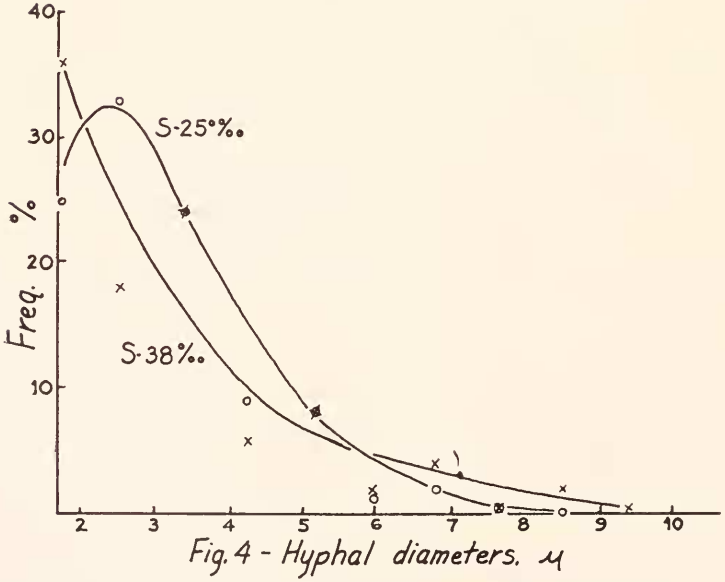

FIGURE 4. Effect of salinity on hyphal diameter. Salinities as indicated; both cultures at 30° C.

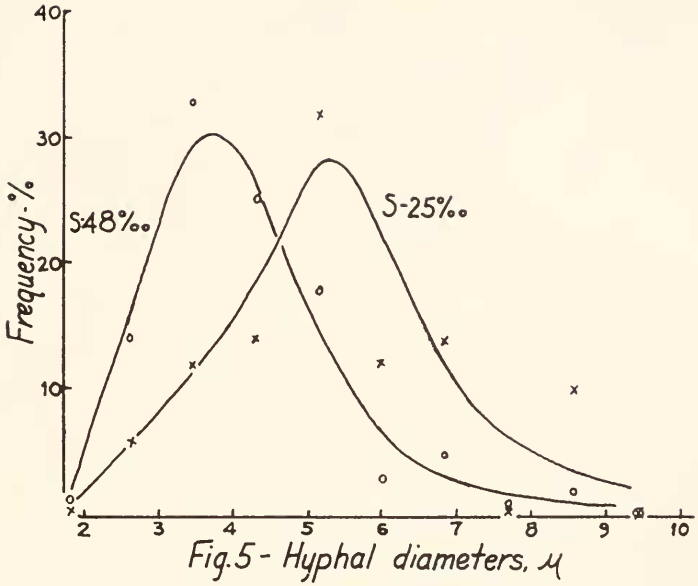

FIGURE 5. Effect of salinity on hyphal diameter. Salinities as indicated; both cultures at 37° C.

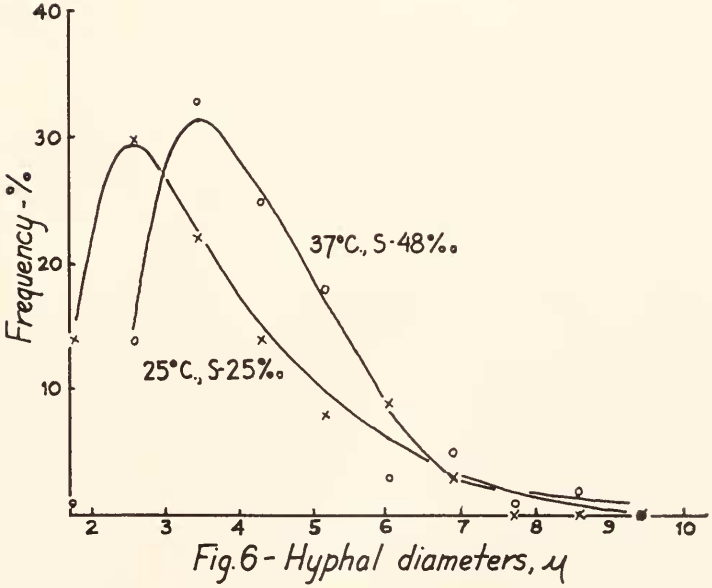

FIGURE 6. Combined effects of salinity and temperature on hyphal diameters. See text for explanation and discussion.

When salinity alone was considered, temperature being held constant, cell diameters were little altered as long as the lower temperatures were being investigated. Thus at 16° C., cultures grown at their apparent optimum for that temperature, *i.e.*, 20 S ‰, yielded data similar to those obtained from cultures from media at 25 S ‰ (Fig. 3). Essentially the same thing can be said for the cultures kept at 30° C. (Fig. 4). (Cultures at 25° C. are not treated here because the optimum at 25° C. is 25 S ‰, which happens to be the one salinity which was used for all the experimental cultures.) When, however, a run was made at 37° C., with one culture at 25 S ‰ and one at the optimal 48 S ‰, the curves show that at this high temperature the salinity of the medium made a difference in the distribution of hyphal diameters, with the lower concentration producing a more nearly "normal" curve, statistically considered, than the optimal concentration. In general, the frequency curves were strongly skewed in the direction of thicker hyphae, with relatively few of the thicker and very few of the thickest ones. Hoffman (1953) obtained similar curves, skewed to the right, in his analysis of cell size of mouse tumors. Such a distribution is not unusual in a sample like this, in which larger cells can exist, but cells below a certain minimum apparently cannot. The cultures in the higher concentration, 48 S ‰, had more narrow hyphae and fewer thick ones, in this respect resembling cultures grown at lower temperatures (Fig. 5).

This last observation is emphasized by comparison of simultaneous alteration of both temperature and salinity. When this is done, one notes a great reduction in the differences that were apparent when either factor alone was raised to a rather high level. Two curves, one from a culture from 25° C. and 25 S ‰ and one from 37° C. and 48 S ‰, are compared in Figure 6. Except for a slight preponderance of thicker hyphae in the culture from the higher temperature, the two curves are very much alike. By and large, simply raising the temperature has little effect upon the cell size of this fungus unless the temperature approaches the maximum possible, at which point cell diameters are generally increased. Similarly, raising the salinity is relatively ineffective unless the divergence in salinity is provided at a high temperature. The effect of either factor can be reduced by concomitant alteration of the other factor.

Discussion

A comparison can be made between two kinds of response of the fungus to temperature and salinity variations. One is expressed as regulation of radial growth of the entire colony, and one as regulation of hyphal diameters. A similarity between the two kinds of response appears in a review of the total temperature range. As long as the colonies are incubated at low or optimal or slightly supra-optimal temperatures, the change in salinity optima with rising temperature is relatively slight, but as the temperature approaches the maximum for the organism, the salinity optimum, broad as it is, becomes much higher. Similarly, hyphal diameters are little affected by a rise in incubation temperature through the lower registers, but as the maximum is approached, the diameters are markedly greater. It should be recalled, however, that if both factors are made to approach their maxima simultaneously, cell size scarcely changes

The Coelenterate, *Cordylophora,* affords a comparable example among animals. Kinne (1958) recorded the sizes of hydranths and the number and size of tentacles in that organism, and found that when the temperature was low (10° C.), growth was greater in fresh than in saline water (30 S ‰), but at 20° C., the salinity relation reversed. This pattern in principle resembles that operating in *Phoma,* but the temperature effects upon ectodermal cell size of the animal were unlike those on the cells of the plant. Animals raised in fresh water showed a temperature effect in which the ten-degree animals had relatively wider cells than the twenty-degree ones; but animals raised in salt water (30 S ‰) showed an opposite reaction. In neither the animal nor the fungus, however, did cell size have a direct relation to total colony size, and the conclusion is evident that total size of the organism is very slightly or not at all dependent upon cell size, and that the mechanism by which temperature and salinity affect total growth is different from the mechanism by which those factors affect the size of individual cells.

Summary

1. When a marine isolant of *Phoma herbarum* West. was grown in a variety of temperatures and on media containing various amounts of salts in the proportions of sea water, the size of the cells, expressed as hyphal diameters, was not greatly changed unless a temperature high enough to be inhibitory (37° C.) was applied in conjunction with a relatively low salinity (25 S ‰), under which conditions more thick hyphae and fewer thin ones grew.

2. Temperature effects upon total growth, expressed as colony diameter, and upon cell size, expressed as hyphal diameters, were similar only in that a temperature approaching the maximum is required to make any considerable change in the salinity optima or in the frequency of larger cells.

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